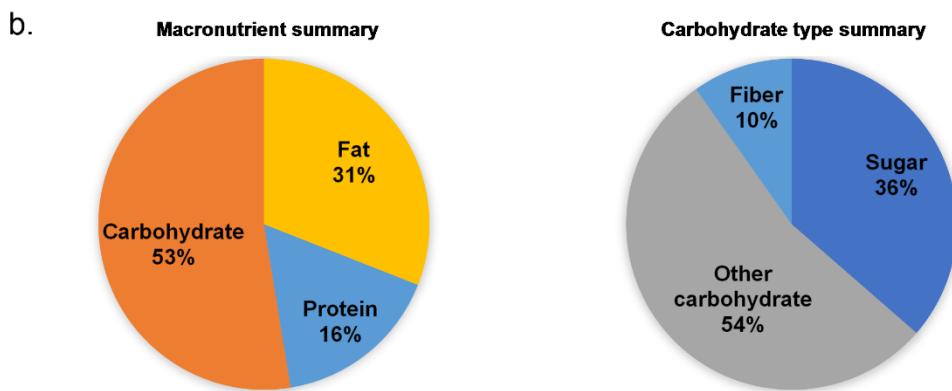
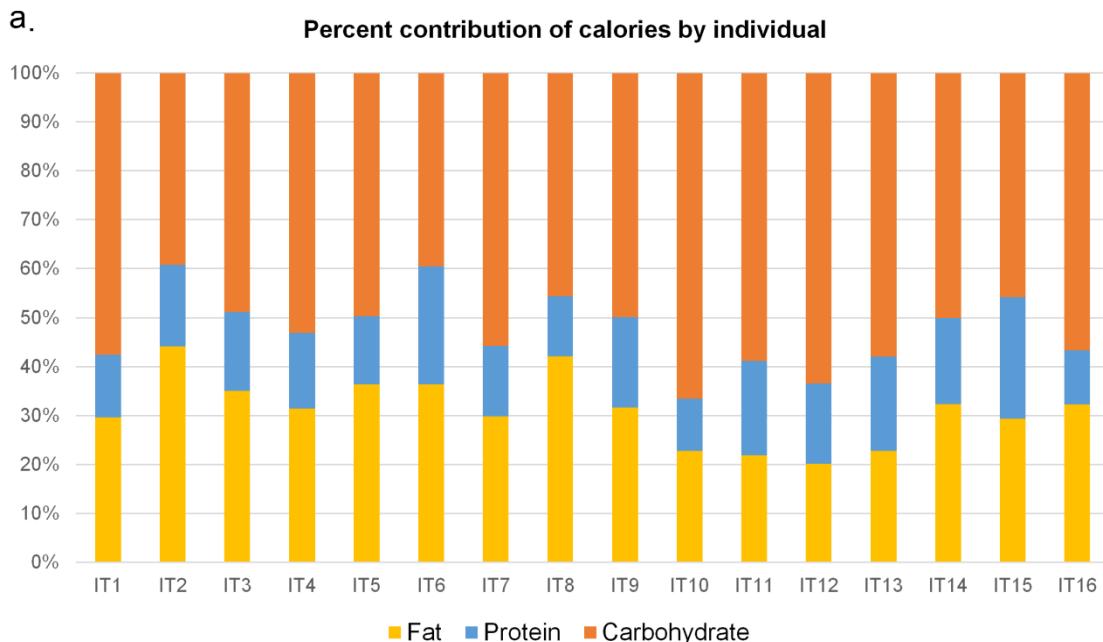


## SUPPLEMENTARY INFORMATION

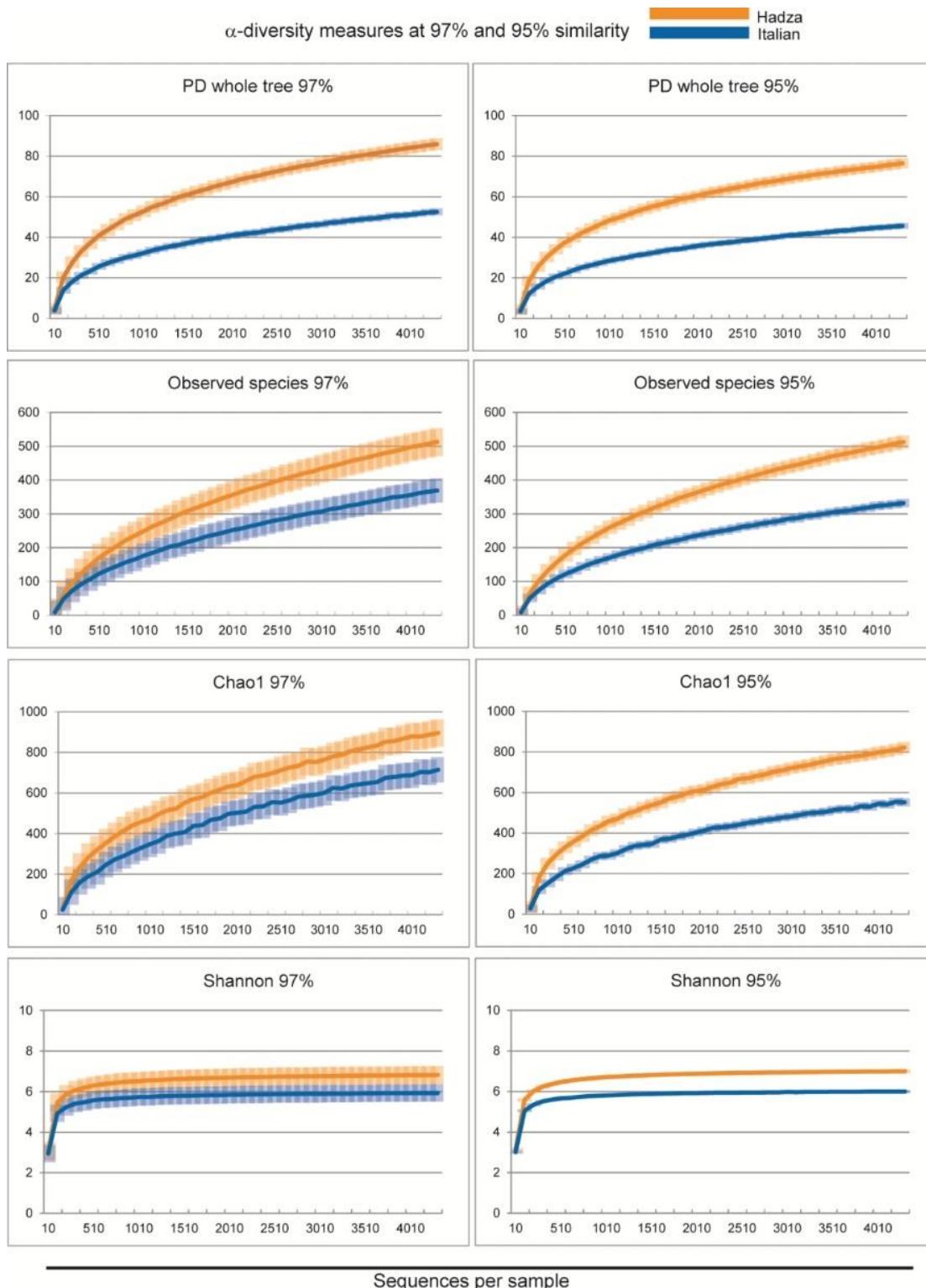
### Figures



**Supplementary Figure 1. Insoluble fiber found in most Hadza tubers.** Tough insoluble fibers make up a large proportion of many frequently consumed of Hadza tubers. Pictured above are two slices of one mak'alitako tuber (*Emminia entennulifa*). The left slice is pre-chewed and the right slice is post-chewing to show the resiliency of the insoluble fiber. These fibers are not swallowed by the Hadza but are instead chewed for a period of one to three minutes, wadged against the cheek and teeth to remove all moisture and pulp, and then expectorated as a “quid”. It is not known if and how much trace amounts of these insoluble fibers do pass into the forager digestive system and inevitably into the colon. Photo by S. L. Schnorr.

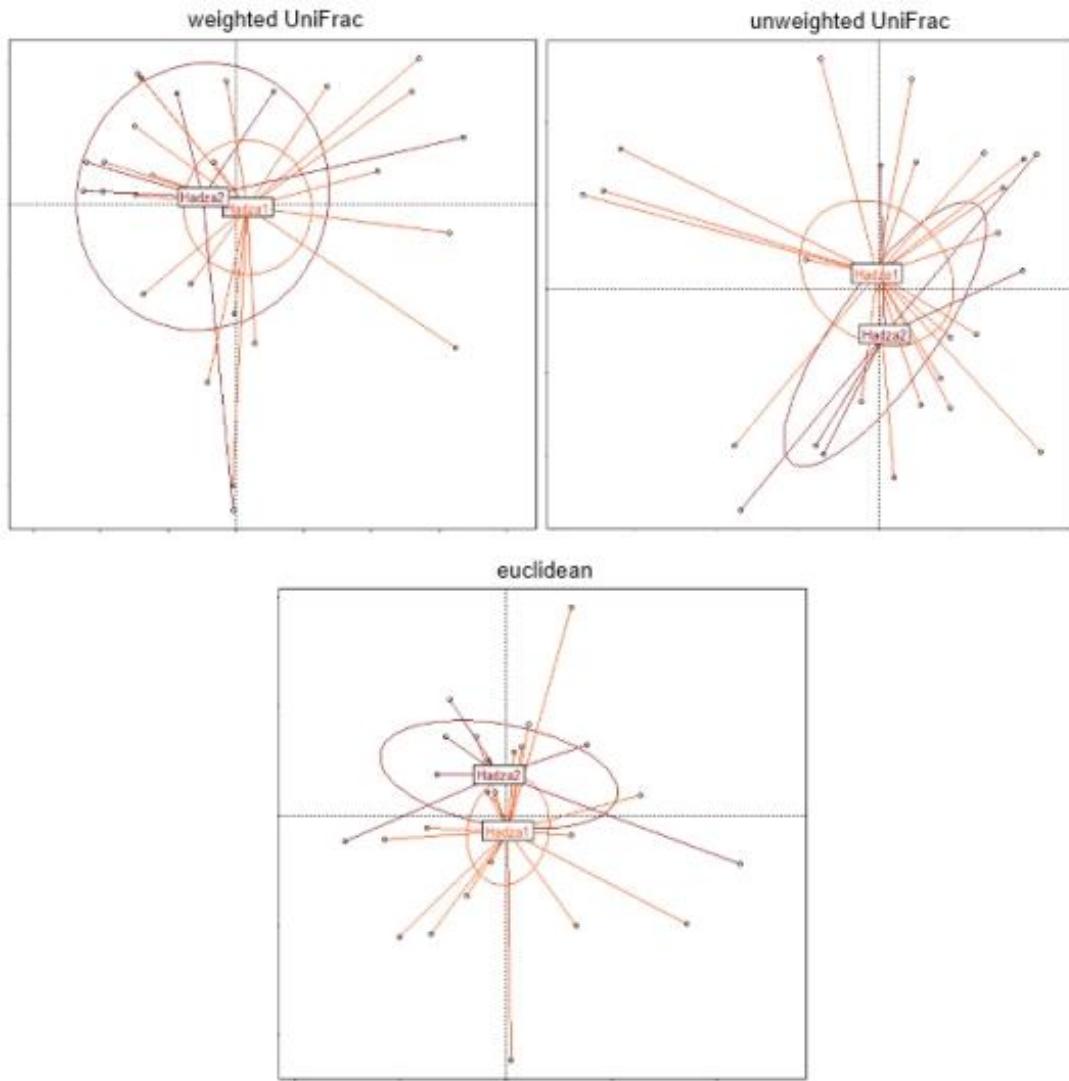


**Supplementary Figure 2. Macronutrient summaries of Italian cohort diet.** (a) The percent macronutrient intake is reported by subject based upon three days of dietary recalls collected during two week days and one weekend day. Recorded diets represent the typical daily diet of each subject. (b) Pie graphs show the summary of the average macronutrient intake by percent of kilocalories consumed for the entire subject cohort as well as a breakdown of carbohydrate type.



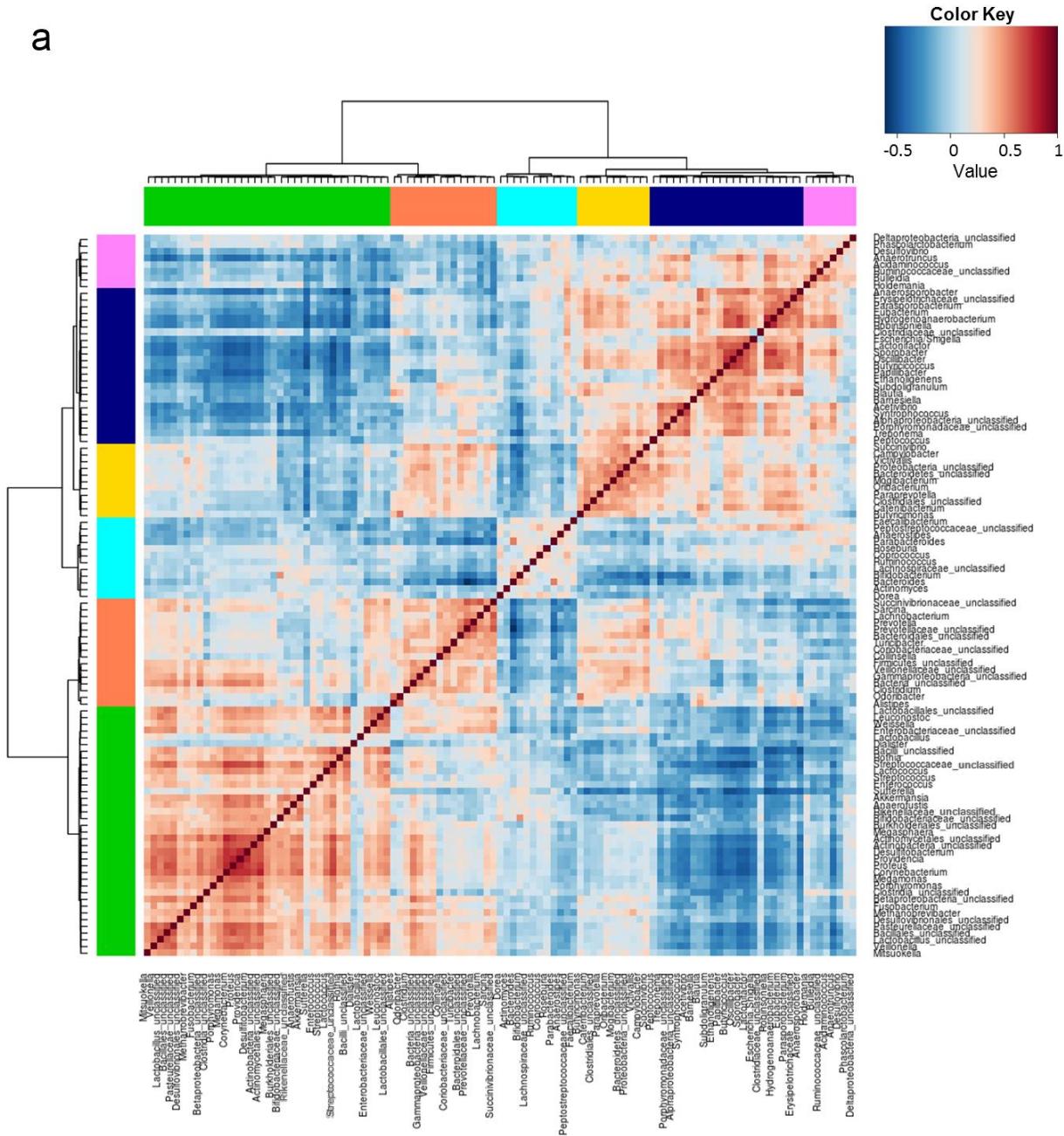
**Supplementary Figure 3. Rarefaction curves of different  $\alpha$ -diversity metrics.** The OTU table at 0.05 and 0.03 similarity thresholds was rarefied up to 4500 reads per sample and analyzed

using various diversity metrics for both Hadza and Italian subjects. Similarity thresholds of 95% (family level) and 97% (genus level) were considered. Metrics used were Faith's Phylogenetic Diversity (PD whole tree), observed OTUs, the Chao1 measure of microbial richness, and the Shannon index of biodiversity. All four diversity metrics showed greater diversity in the Hadza microbiota compared to the Italian microbiota. Plateaus occurred by about 4000 reads per sample, approximating a saturation phase and indicating that the breadth of diversity has been well captured in the sequencing.

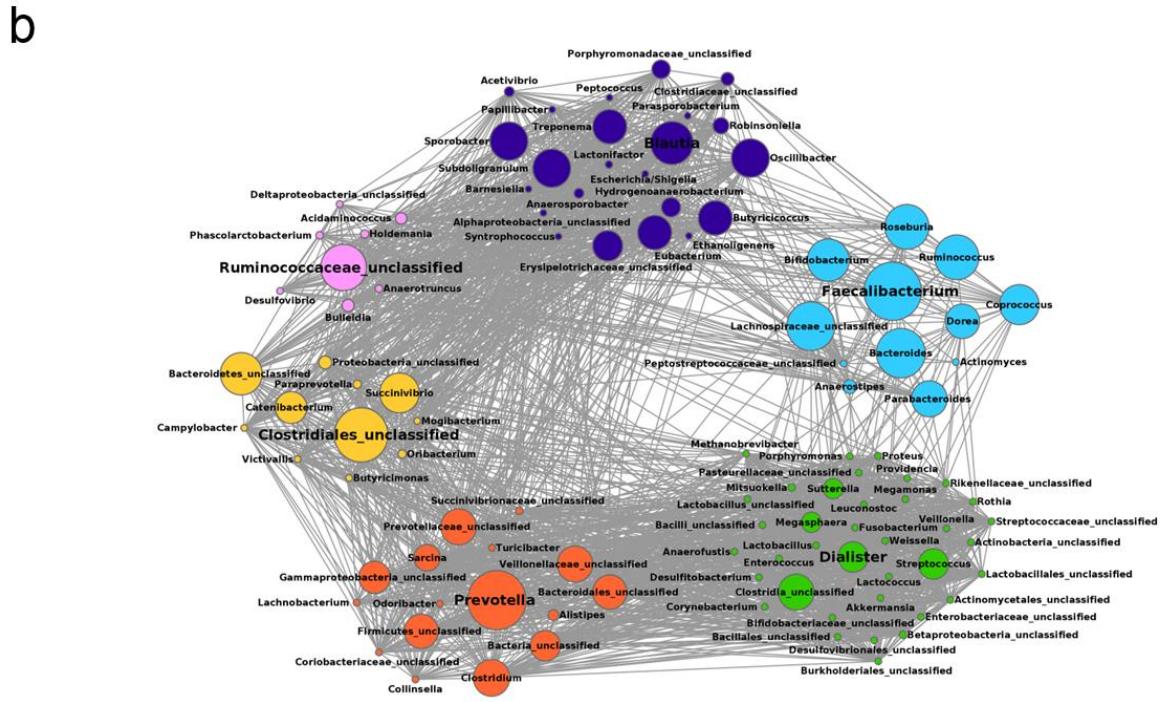


**Supplementary Figure 4. Phylogenetic diversity within the Hadza gut microbial community.** PCoA plots based on weighted and unweighted UniFrac distances and Euclidean distances of the genus level relative abundances are shown. Subjects are grouped according to camp provenience, Dedauko (Hadza 1) and Sengele (Hadza 2). None of the diversity metrics show significant separation of the Hadza gut microbiota by camp location.

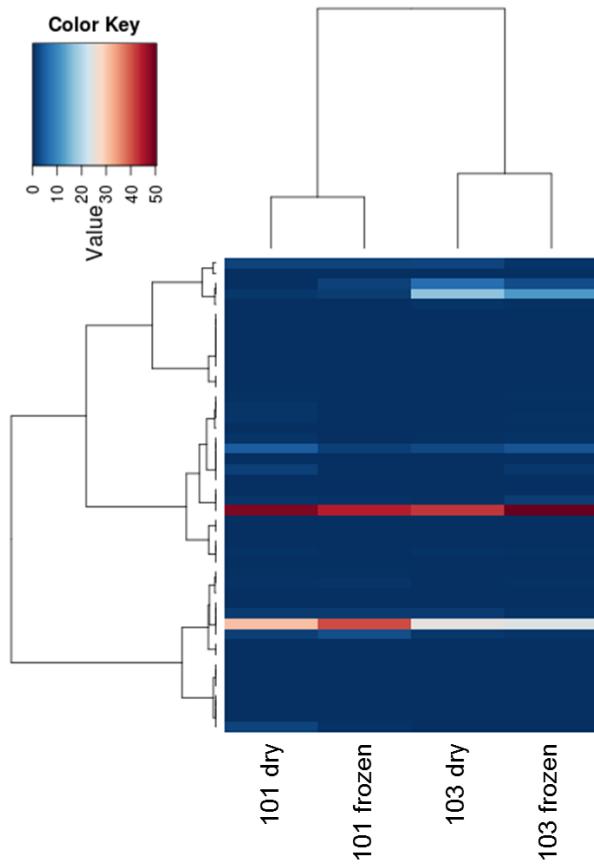
## Supplementary Figure 5. Assignments of bacterial co-abundance groups (CAGs)



## Supplementary Figure 5. Assignments of bacterial co-abundance groups (CAGs)



**Supplementary Figure 5. Assignments of bacterial co-abundance groups (CAGs).** CAG assignment relied on a heat plot (**a**) showing Kendall correlations between genera clustered by the Spearman correlation coefficient and Ward linkage hierarchical clustering. Colors are indicative of the six identified CAGs. (**b**) Wiggum plot correlations between the six CAGs identified, circle size is representative of the genus abundance and the connections between nodes represent positive and significant Kendall correlations between genera (FDR < 0.05).



**Supplementary Figure 6. Comparison of dried and frozen storage protocols on test fecal samples.** In test experiments, stools from two Western individuals (101 and 103) were collected and split into two samples each. One fraction was stored immediately at -80 °C (frozen) and the second fraction was dried following a two-step ethanol submersion and silica storage procedure (dried). Hierarchical ward-linkage clustering based on the Spearman correlation coefficients of bacterial family proportion is shown. Subjects are clustered in the top of the panel. Families are clustered by the vertical tree.

## Tables

**Supplementary Table 1. Summary of Hadza foods and nutrition.**

					g 100g <sup>-1</sup> dry wt.					kcal 100g <sup>-1</sup> dry wt.	
Food type	Hadza name	English name	Species name	Moisture (%)	Edible portion	Carbohydrate	Protein	Fat	Fiber	Energy	References
<b>Baobab</b>	N//obabe (pulp)**	Baobab	<i>Adansonia digitata</i>	4.7	na	46.6	2.5	0.7	45.1	203.0	1
	N//obabe (seed)**	Baobab	<i>Adansonia digitata</i>	4.8	na	11.2	36.3	29.3	14.1	454.0	1
<b>Berry</b>	Kisinubi	na	<i>Cordia sinensis</i>	73.0	na	68.8	12.6	1.8	11.6	342.0	1
	Undushibi**	na	<i>Cordia sinensis</i>	71.0	na	61.4	15.2	na	13.6	324.0	1
<b>Masakapi</b>	Masakapi	na	<i>Cordia crenata</i>	69.0	na	62.5	12.7	1.9	17.8	318.0	1
	Hlukayebé	na	<i>Grewia villosa</i>	24.0	na	72.7	7.1	na	13.4	337.0	1
<b>Kongolubi</b>	Kongolubi	na	<i>Grewia bicolor</i>	26.0	na	66.1	12.0	2.0	13.2	330.0	1
	Pawe	na	<i>Sclerocarya birrea</i>	83.0	na	49.9	3.6	na	37.7	232.0	1
<b>Honey</b>	Ba'alako**	Honey (stinging)	<i>Apis mellifera</i>	15.1	na	89.1	3.3	7.2	na	434.0	1
	N//ateko**	Honey (stingless)	<i>Meliponinae sp.</i>	23.5	na	92.8	3.2	3.2	na	412.5	1
<b>Meat</b>	Gewedako**	Dik-dik	<i>Madoqua kirkii</i>	na	na	na	na	na	na	na	2
	Tsokwonako**	Giraffe	<i>Giraffa camelopardalis</i>	na	na	na	na	na	na	na	2
<b>Chacha**</b>	Chacha**	Galago (lesser)	<i>Galago senegalensis</i>	na	na	na	na	na	na	na	2
	Ndonoko**	Galago (greater)	<i>Otolemur crassicaudatus</i>	na	na	na	na	na	na	na	2
<b>Tuber</b>	Tsunako**	Bee larvae	<i>Apis mellifera</i>	na	na	na	na	na	na	na	2
	//Ekwa hasa**	na	<i>Vigna frutescens</i>	68.8	39.4	60.8	7.2	0.8	28.2	282.3	3
<b>//Ekwa gadabi</b>	//Ekwa gadabi	na	<i>Vigna frutescens</i>	70.0	na	60.6	8.0	1.9	22.7	84.5	4
	Shumuko**	na	<i>Vatoraea pseudolablab</i>	90.1	na	50.8	3.3	0.6	35.2	225.0	4
<b>Do'aiko</b>	Do'aiko	na	<i>Vigna macrorhyncha</i>	85.0	na	58.6	10.4	0.1	22.4	292.0	4
	Matukwaiko	na	<i>Coccinea surantiaca</i>	86.5	na	69.5	12.4	0.0	13.3	337.0	4
<b>Mak'alitako**</b>	Mak'alitako**	na	<i>Eminia entennulifa</i>	71.7	56.9	26.4	4.1	1.1	17.1	110.1	3, 4
	Panjuko**	na	<i>Ipomoea transvaalensis</i>	70.6	91.8	54.8	4.2	1.7	2.6	233.0	4

**Supplementary Table 2. Percent contribution of food categories and corresponding nutrients to average daily Italian cohort diet.**

Food Category	Most common food items	Energy (kcal)	Fat (g)	Protein (g)	All carbohydrate (g)	Fiber (g)	Sugar (g)	Other carbohydrate (g)
<b>Bread, cereal, pasta, grain</b>		37.2%	25.3%	33.6%	48.3%	45.2%	16.0%	74.0%
	flatbread, brioche, brown bread							
	pasta: tortellini, lasagna, noodles							
	cereals, biscuits, crackers, pizza crust							
<b>Other starch</b>		3.7%	1.2%	2.3%	5.7%	6.3%	0.7%	8.5%
	beans, legumes							
	rice, potatoes, couscous							
<b>Meats, egg, meat broths</b>		10.7%	16.0%	31.2%	0.7%	0.4%	1.0%	0.5%
	pork: cured, steak, roast, salami							
	chicken: baked, roasted							
	beef: steak, roast, sausage,							
	fish: tuna, sole, cod, bass, flounder							
<b>Dairy</b>		13.0%	22.3%	19.9%	5.1%	0.5%	13.0%	0.7%
	milk, cream, yogurt							
	cheese: parmesan, mozzarella, sheep							
<b>Fruit, fruit juice</b>		8.8%	0.8%	2.4%	17.2%	19.9%	29.4%	5.6%
	banana, apple, pear, persimmon, citrus							
	orange juice, pear juice							
<b>Vegetables, sauces, herbs</b>		4.6%	3.1%	5.4%	6.1%	23.1%	4.9%	4.6%
	lettuce, carrot, onion							
	tomato sauce, mixed vegetables							
	boiled cabbage, squash							
<b>Desserts, sugary treats</b>		12.6%	14.6%	4.0%	14.6%	2.8%	33.3%	4.8%

	sugar, honey, cake, jam, soda							
	chocolate, pastries, ice cream							
<b>Oil, butter, fat</b>		4.4%	13.6%	0.0%	0.0%	0.0%	0.0%	0.0%
	olive oil, butter							
<b>Alcohol</b>		3.5%	0.0%	0.3%	1.4%	0.0%	0.3%	0.8%
	beer, wine							
<b>Nuts</b>		1.1%	3.1%	0.8%	0.2%	1.2%	0.1%	0.2%
	walnut, peanut							
<b>Other</b>		0.4%	0.1%	0.1%	0.6%	0.5%	1.3%	0.2%
	espresso, coffee, barley coffee, tea							
	vinegar, mustard, salt							
<b>Average daily amount</b>		<b>1852.3</b>	<b>66.3</b>	<b>75.0</b>	<b>236.5</b>	<b>20.0</b>	<b>84.5</b>	<b>120.6</b>

Percent contribution of each food group for energy (kcal), fat (g), protein (g), and carbohydrate (g). Carbohydrate is further broken down into types of carbohydrate with percent contribution by gram of the following: fiber, sugar, and other carbohydrate (oligosaccharides). An average daily intake for each category is reported in the last row.

**Supplementary Table 3. Age, sex and provenience of study enrolled participants.**

HADZA			
SAMPLE ID	AGE	SEX	CAMP
H1	43	F	Dedauko
H2	37	M	Dedauko
H3	22	M	Dedauko
H4	24	M	Dedauko
H5	70	F	Dedauko
H6	30	M	Dedauko
H7	38	F	Dedauko
H8	20	F	Dedauko
H9	47	F	Dedauko
H10	40	F	Dedauko
H11	29	F	Dedauko
H12	8	F	Dedauko
H13	34	M	Dedauko
H14	30	M	Dedauko
H15	16	M	Dedauko
H16	21	M	Dedauko
H17	23	M	Dedauko
H18	17	M	Dedauko
H19	15	M	Dedauko
H20	13	M	Dedauko
H21	43	M	Sengele
H22	38*	M	Sengele
H23	24*	M	Sengele
H24	65*	M	Sengele
H25	40*	M	Sengele
H26	48*	M	Sengele
H27	29	F	Sengele

ITALIANS			
SAMPLE ID	AGE	SEX	PROVENIENCE
IT1	38	M	Bologna
IT2	34	F	Bologna
IT3	29	F	Bologna
IT4	27	M	Bologna
IT5	30	M	Bologna
IT6	32	F	Bologna
IT7	25	F	Bologna
IT8	34	M	Bologna
IT9	39	F	Bologna
IT10	40	F	Bologna
IT11	40	F	Bologna
IT12	30	F	Bologna
IT13	30	F	Bologna
IT14	21	F	Bologna
IT15	29	M	Bologna
IT16	32	F	Bologna

Asterisks indicate individuals for whom age was less certain.

**Supplementary Table 4. Summary of taxa differences between Hadza and Italian subjects.**

**a**

over-represented in Hadza					
Phylum	Italians ± SEM		Hadza ± SEM		FDR
Bacteroidetes	10.1	2.7	17.4	1.1	0.002
Bacteria unclassified	0.2	0.05	1.8	0.2	< 0.001
Proteobacteria	0.3	0.04	5.9	1.2	< 0.001
Spirochaetes	0	0	2.8	0.9	< 0.001
Genus					
<i>Anaerophaga</i>	0	0	0.6	0.1	< 0.001
Bacteroidales unclassified	0.07	0.02	0.8	0.1	< 0.001
<i>Porphyromonadaceae</i> unclassified	0.03	0.02	0.9	0.3	< 0.001
<i>Prevotella</i>	0.4	0.3	6.2	1.1	< 0.001
Bacteroidetes unclassified	0.2	0.07	6.6	0.8	< 0.001
Sphingobacteriales unclassified	0	0	0.6	0.1	< 0.001
<i>Eubacterium</i>	1.4	0.6	2.2	0.5	0.013
<i>Robinsoniella</i>	0.02	0	0.9	0.2	< 0.001
Clostridiales unclassified	7.4	0.8	11.1	0.6	< 0.001
<i>Butyriviboccus</i>	0.4	0.05	2.6	0.5	< 0.001
<i>Hydrogenoanaerobacterium</i>	0.07	0.03	1	0.1	< 0.001
<i>Oscillibacter</i>	1	0.3	3.8	0.4	< 0.001
<i>Ruminococcaceae</i> unclassified	3.6	0.6	8.6	0.7	< 0.001
<i>Sporobacter</i>	0.8	0.2	3.7	0.4	< 0.001
<i>Veillonellaceae</i> unclassified	0.02	0.01	0.8	0.2	< 0.001
<i>Bulleidia</i>	0	0	0.7	0.07	< 0.001
<i>Catenibacterium</i>	0.7	0.4	1.4	0.4	0.001
Firmicutes unclassified	0.3	0.1	0.8	0.1	< 0.001
<i>Ruminobacter</i>	0	0	1.2	0.7	0.045
<i>Succinivibrio</i>	0.02	0.02	2.8	0.7	< 0.001
Proteobacteria unclassified	0.02	0.01	0.5	0.2	< 0.001
<i>Treponema</i>	0	0	2.5	0.7	< 0.001

**b**

over-represented in Italians					
Phylum	Italians ± SEM		Hadza ± SEM		FDR
Actinobacteria	8.3	1.5	0.1	0.02	< 0.001
Firmicutes	80.9	2.7	71.8	1.9	0.006
Genus					
<i>Bifidobacterium</i>	8.1	1.5	0.02	0.01	< 0.001
<i>Bacteroides</i>	7.1	2	0.2	0.04	< 0.001
<i>Alistipes</i>	0.9	0.2	0	0	< 0.001
<i>Blautia</i>	9.5	1.3	3.5	0.4	< 0.001

<i>Anaerosporobacter</i>	0.6	0.1	0.2	0.04	0.003
<i>Coprococcus</i>	3.6	0.7	1.4	0.2	0.005
<i>Dorea</i>	1.4	0.2	0.4	0.06	< 0.001
<i>Lachnospiraceae unclassified</i>	6.4	0.7	3.9	0.2	0.001
<i>Roseburia</i>	7.7	1.3	3.9	0.8	0.014
<i>Faecalibacterium</i>	18.5	2.4	11.8	1	0.022
<i>Ruminococcus</i>	8.6	1.8	2.1	0.3	0.005
<i>Erysipelotrichaceae unclassified</i>	1.5	0.3	0.7	0.2	0.026

Bacterial phyla and genera significantly over-represented in the gut ecosystem of Hadza (**a**) and Italians (**b**). For each microbial group, the mean relative abundance (%)  $\pm$  SEM and *P*-value of the differences between the two populations (Mann-Whitney U test) are reported.

**Supplementary Table 5. qPCR quantification of *Bifidobacterium* in stool samples.**

<b>Hadza</b>		<b>Italians</b>	
Subject	16S rRNA gene copies ng <sup>-1</sup> faecal DNA	Subject	16S rRNA gene copies ng <sup>-1</sup> faecal DNA
H1	BDL	IT1	6.55E+05
H2	BDL	IT2	1.41E+06
H3	BDL	IT3	1.39E+06
H4	BDL	IT4	1.38E+04
H5	BDL	IT5	1.19E+06
H6	BDL	IT6	2.05E+05
H7	BDL	IT7	1.22E+05
H8	BDL	IT8	1.88E+05
H9	BDL	IT9	1.50E+05
H10	BDL	IT10	6.55E+04
H11	BDL	IT11	7.53E+04
H12	BDL	IT12	5.48E+05
H13	BDL	IT13	2.36E+05
H14	BDL	IT14	1.56E+05
H15	BDL	IT15	1.82E+05
H16	BDL	IT16	1.30E+05
H17	BDL		
H18	BDL		
H19	BDL		
H20	BDL		
H21	BDL		
H22	BDL		
H23	BDL		
H24	BDL		
H25	BDL		
H26	BDL		
H27	BDL		
H28	BDL		

For each subject, the *Bifidobacterium* 16S rRNA copy number  $\mu\text{g}^{-1}$  of fecal DNA is reported.

BDL: Below detection limit (100 16S rRNA gene copies ng<sup>-1</sup> faecal DNA)

**Supplementary Table 6. Absolute and percentage based values of SCFAs in Hadza and Italian samples.**

**a**

HADZA	Acetic (umol g <sup>-1</sup> )	Propionic (umol g <sup>-1</sup> )	Butyric (umol g <sup>-1</sup> )	Valeric (umol g <sup>-1</sup> )
H16	1.4	0.27	0.12	0.025
H1	2.3	0.71	0.33	0.063
H14	2.7	1.06	0.79	0.104
H2	2.3	0.67	0.69	0.119
H13	4.7	1.71	1.26	0.126
H11	4.9	1.81	1.19	0.141
H12	4.3	1.90	1.65	0.181
H10	4.8	1.38	0.71	0.108
H15	2.4	0.98	0.77	0.114
H6	3.5	1.46	0.80	0.070
H5	5.2	0.96	0.32	0.091
H4	1.8	0.52	0.33	0.096
H8	4.5	1.40	0.57	0.075
H7	4.0	1.55	0.70	0.098
H3	4.8	1.01	0.67	0.129
H9	4.3	0.98	0.44	0.063
H22	1.9	0.39	0.27	0.043
H19	2.2	0.50	0.41	0.054
H17	4.5	2.00	1.79	0.177
H21	3.4	0.71	0.48	0.063
H20	3.4	1.09	0.23	0.061
H18	2.2	0.56	0.36	0.058
H23	1.5	0.40	0.34	0.040
H25	2.2	0.87	0.67	0.082
H24	3.9	1.85	0.63	0.075
H26	0.8	0.17	0.13	0.027
H28	0.8	0.17	0.06	0.021
H27	0.8	0.17	0.11	0.021

**b**

ITALIANS	Acetic (umol g <sup>-1</sup> )	Propionic (umol g <sup>-1</sup> )	Butyric (umol g <sup>-1</sup> )	Valeric (umol g <sup>-1</sup> )

IT11	1.1	0.43	0.20	not determined
IT1	9.5	2.20	2.64	0.168
IT6	2.2	0.41	0.65	0.045
IT3	3.1	0.75	1.52	0.075
IT5	1.7	0.39	0.30	0.083
IT2	1.1	0.10	0.13	0.039
IT4	0.7	0.17	0.18	0.030
IT12	1.7	0.25	0.46	0.021
IT8	1.4	0.40	0.36	0.038
IT14	0.4	0.06	0.05	0.010
IT10	1.3	0.34	0.35	0.048
IT7	3.5	0.71	1.20	0.056
IT16	1.8	0.58	0.20	0.003
IT15	2.9	0.68	0.81	0.076
IT9	0.7	0.17	0.22	0.024
IT13	5.2	0.91	1.53	0.068

c

HADZA	TOTAL ( $\mu\text{mol g}^{-1}$ )	Acetic	Propionic	Butyric	Valeric
		%	%	%	%
H16	1.83	77.23	14.56	6.82	1.39
1H	3.42	67.89	20.76	9.53	1.83
H14	4.69	58.34	22.55	16.90	2.21
H2	3.77	60.71	17.80	18.34	3.15
H13	7.80	60.23	21.96	16.19	1.62
H11	8.04	60.95	22.51	14.78	1.76
H12	8.07	53.86	23.51	20.39	2.24
H10	6.96	68.38	19.80	10.25	1.56
H15	4.31	56.67	22.81	17.89	2.64
H6	5.84	60.17	24.92	13.72	1.19
H5	6.53	79.01	14.74	4.85	1.40
H4	2.74	65.63	18.91	11.97	3.50
H8	6.57	68.95	21.24	8.67	1.14
H7	6.34	62.93	24.43	11.09	1.55
H3	6.60	72.57	15.37	10.10	1.96
H9	5.76	74.32	17.03	7.56	1.09
H22	2.63	73.07	15.01	10.27	1.65

H19	3.15	69.40	15.96	12.92	1.71
H17	8.42	52.87	23.75	21.27	2.11
H21	4.66	73.02	15.27	10.36	1.35
H20	4.81	71.35	22.55	4.83	1.27
H18	3.18	69.06	17.73	11.38	1.83
H23	2.24	65.24	17.80	15.20	1.76
H25	3.85	57.66	22.70	17.52	2.13
H24	6.43	60.27	28.81	9.76	1.16
H26	1.12	70.57	15.22	11.79	2.42
H28	1.07	76.45	16.08	5.45	2.01
H27	1.06	71.64	15.62	10.75	1.99

**d**

ITALIANS	TOTAL ( $\mu\text{mol g}^{-1}$ )	Acetic	Propionic	Butyric	Valeric
		%	%	%	%
IT11	1.75	63.59	24.79	11.62	not determined
IT1	14.55	65.55	15.13	18.16	1.16
IT6	3.27	66.11	12.68	19.85	1.36
IT3	5.43	56.81	13.89	27.93	1.37
IT5	2.50	69.04	15.49	12.15	3.33
IT2	1.35	79.97	7.52	9.62	2.89
IT4	1.09	65.64	15.26	16.36	2.74
IT12	2.46	70.62	10.00	18.53	0.85
IT8	2.23	63.95	18.10	16.24	1.70
IT14	0.49	73.59	13.20	11.13	2.07
IT10	2.08	64.58	16.45	16.66	2.31
IT7	5.52	64.22	12.90	21.86	1.03
IT16	2.58	69.77	22.38	7.72	0.13
IT15	4.49	65.13	15.18	18.00	1.68
IT9	1.09	62.53	15.34	19.94	2.19
IT13	7.72	67.44	11.84	19.84	0.88

(a) Absolute ( $\mu\text{mol g}^{-1}$ ) amount of acetic, propionic, butyric, and valeric acids in fecal samples from Hadza and (b) Italians. (c) Relative (%) amount of acetic, propionic, butyric, and valeric acids in fecal samples from Hadza and (d) Italians.

**Supplementary Table 7. Comparison of dried and frozen storage protocols on test fecal samples.**

**a**

Sample ID	DNA ng $\mu\text{l}^{-1}$	A260	A280	260/280	260/230
101 frozen	423.86	8.477	4.494	1.89	1.95
101 dry	610.86	12.217	6.299	1.94	2.06
103 frozen	468.69	9.374	5.213	1.8	1.39
103 dry	260.74	5.215	2.879	1.81	1.54

**b**

Sample	Acetic acid (%)	Propionic acid (%)	Butyric acid (%)	Valeric acid (%)
101 frozen	77.23	14.56	6.82	1.39
101 dry	80.45	11.17	7.44	0.94
103 frozen	67.89	20.76	9.53	1.83
103 dry	72.60	18.58	7.87	0.94

(a) Total DNA yield and absorbance values, and (b) SCFA relative abundance profiles are shown for comparison.

## **Supplementary References**

1. Murray, S. S., Schoeninger, M. J., Bunn, H. T., Pickering, T. R. & Marlett, J. A. Nutritional Composition of Some Wild Plant Foods and Honey Used by Hadza Foragers of Tanzania. *Journal of Food Composition and Analysis* **14**, 3–13 (2001).
2. Marlowe, F. W. *The Hadza: Hunter-Gatherers of Tanzania*. 336 (University of California Press: London, 2010).
3. Schoeninger, M. J., Bunn, H. T., Murray, S. S. & Marlett, J. A. Composition of Tubers Used by Hadza Foragers of Tanzania. *Journal of Food Composition and Analysis* **14**, 15–25 (2001).
4. Vincent, A. S. Plant foods in savanna environments: a preliminary report of tubers eaten by the Hadza of Northern Tanzania. *World archaeology* **17**, 131–48 (1985).